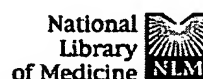


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PCR amplification on a microarray of gel-immobilized oligonucleotides: detection of bacterial toxin- and drug-resistant genes and their mutations.

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Strizhkov BN, Drobyshev AL, Mikhailovich VM, Mirzabekov AD.

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia.

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PCR amplification on a microarray of gel-immobilized primers (microchip) has been developed. One of a pair of PCR primers was immobilized inside a separate microchip polyacrylamide porous gel pad of 0.1 x 0.1 x 0.02 (or 0.04) micron in size and 0.2 (or 0.4) nL in volume. The amplification was carried out simultaneously both in solution covering the microchip array and inside gel pads. Each gel pad contained the immobilized forward primers, while the fluorescently labeled reverse primers, as well as all components of the amplification reaction, diffused into the gel pads from the solution. To increase the amplification efficiency, the forward primers were also added into the solution. The kinetics of amplification was measured in real time in parallel for all gel pads with a fluorescent microscope equipped with a charge-coupled device (CCD) camera. The accuracy of the amplification was assessed by using the melting curves obtained for the duplexes formed by the labeled amplification product and the gel-immobilized primers during the amplification process; alternatively, the duplexes were produced by hybridization of the extended immobilized primers with labeled oligonucleotide probes. The on-chip amplification was applied to detect the anthrax toxin genes and the plasmid-borne beta-lactamase gene responsible for bacterial ampicillin resistance. The allele-specific type of PCR amplification was used to identify the Shiga toxin gene and discriminate it from the Shiga-like one. The genomic mutations responsible for rifampicin resistance of the Mycobacterium tuberculosis strains were detected by the same type of PCR amplification of the rpoB gene fragment isolated from sputum of tuberculosis patients. The on-chip PCR amplification has been shown to be a rapid, inexpensive and powerful tool to test genes responsible for bacterial toxin production and drug resistance, as well as to reveal point nucleotide mutations.

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